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Effects on Fish of Effluents and Flocculants from Coal Mine Waste Water

Alberta
ENVIRONMENTAL CENTRE

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EFFECTS ON FISH OF EFFLUENTS AND FLOCCULANTS FROM COAL MINE WASTE WATER

Alberta Environmental Centre

Vegreville, Alberta

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ABSTRACT

The purpose of this study was to describe the toxicity to fish of effluents and flocculating agents used at the Coal Valley Mine of Luscar Sterco (1977) Ltd. Toxicity of the final effluent was determined using 96h LC_{50} rainbow trout bioassays at 4 and 15°C. At the end of each test, fish were subjected to gross and histopathological examination to determine specific effects of the effluents. Flocculants (Anionic 8184, Ferric Sulfate, Cationic 8852, Cationic 577C, Cationic LT31) were applied in varying concentration combinations to three dilution waters (mine influent, mine effluent, treated municipal water). Toxicity was measured using 96h LC_{50} rainbow trout bioassays followed by gross and histopathology examination. Based on these experiments, it can be concluded that:

i) Final effluent discharged by the mine was not acutely toxic to fish at either temperature. After 96h of exposure, no abnormalities were found in the fish.

ii) The 96h LC_{50} of the flocculants in mine influent were approximately: Anionic 8184, >300 mg/ℓ; Ferric Sulfate, >125 mg/ℓ; Cationic 8852, >50 mg/ℓ; Cationic 577C, >40 mg/ℓ; Cationic LT31 >40 mg/ℓ. These can be considered maximum dosages that can be applied without being acutely toxic to fish.

iii) All flocculants were less toxic in mine influent with a high suspended solids load than in treated municipal water with a low suspended load.

iv) The major finding of pathological significance related to the necrosis and sloughing of gill epithelial cells and focal degeneration and necrosis of the skin epithelial cells in fish exposed to Cationic flocculants.

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1. INTRODUCTION

1.1 Purpose of Study

In recent years the coal mining industry in Alberta and elsewhere has used progressively greater amounts of chemical flocculants* to treat mine and runoff waters for sediment removal. Although many of these flocculants have been extensively tested by suppliers to determine the concentrations at which they are toxic to fish, some are relatively new and sufficient experimental data are not available to assess their toxicity. Coal Valley Mine, operated by Luscar Sterco (1977) Ltd., is situated in the foothills of the Rocky Mountains, approximately 100 km southwest of Edson (Figure 1). Coal is mined from a series of pits located along the Lovett River Valley (Figure 2). Wastewater from these pits, as well as surface runoff is diverted to a number of impoundments. Flocculants are added to some impoundments to reduce the high suspended solid load prior to discharge to the Lovett River. In 1982 the quality of discharge waters decreased due, in part, to mine expansion through new geologic materials.

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*Flocculant is a compound which induces the coalescing of particles by changing the ionic environment and nature of the particles.

As a result, the effectiveness of flocculants formerly applied to the waste treatment ponds decreased.

In order to improve effluent quality, Coal Valley Mine evaluated a large number of flocculating agents for their effectiveness in suspended solids removal. The most promising combinations of the flocculants evaluated are:

i) ferric sulfate/anionic 8184

ii) cationic 8852/anionic 8184

iii) cationic 577C/anionic 8184

iv) cationic LT31/anionic 8184.

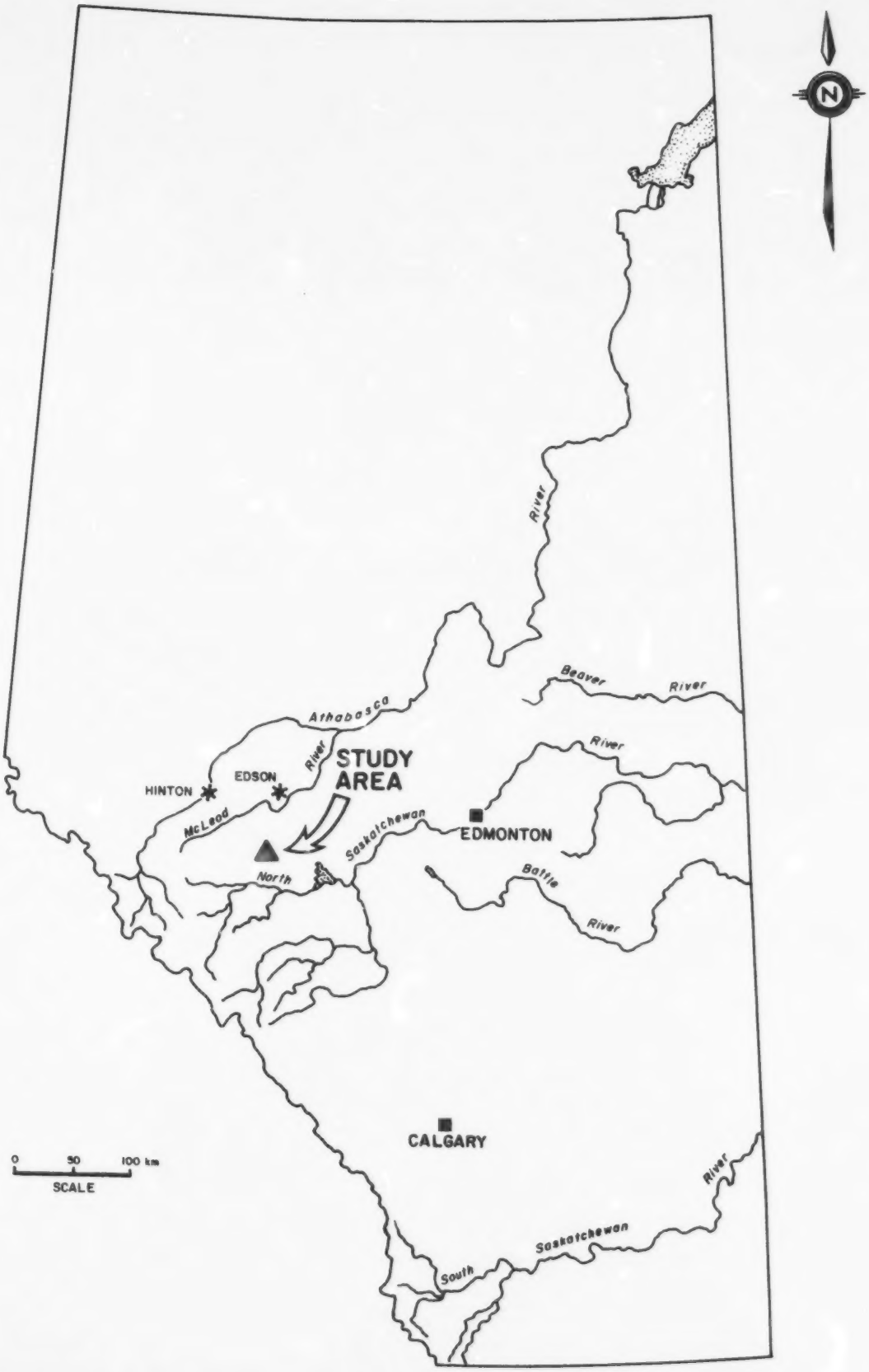


FIGURE 1. MAP OF ALBERTA SHOWING LOCATION OF STUDY AREA.

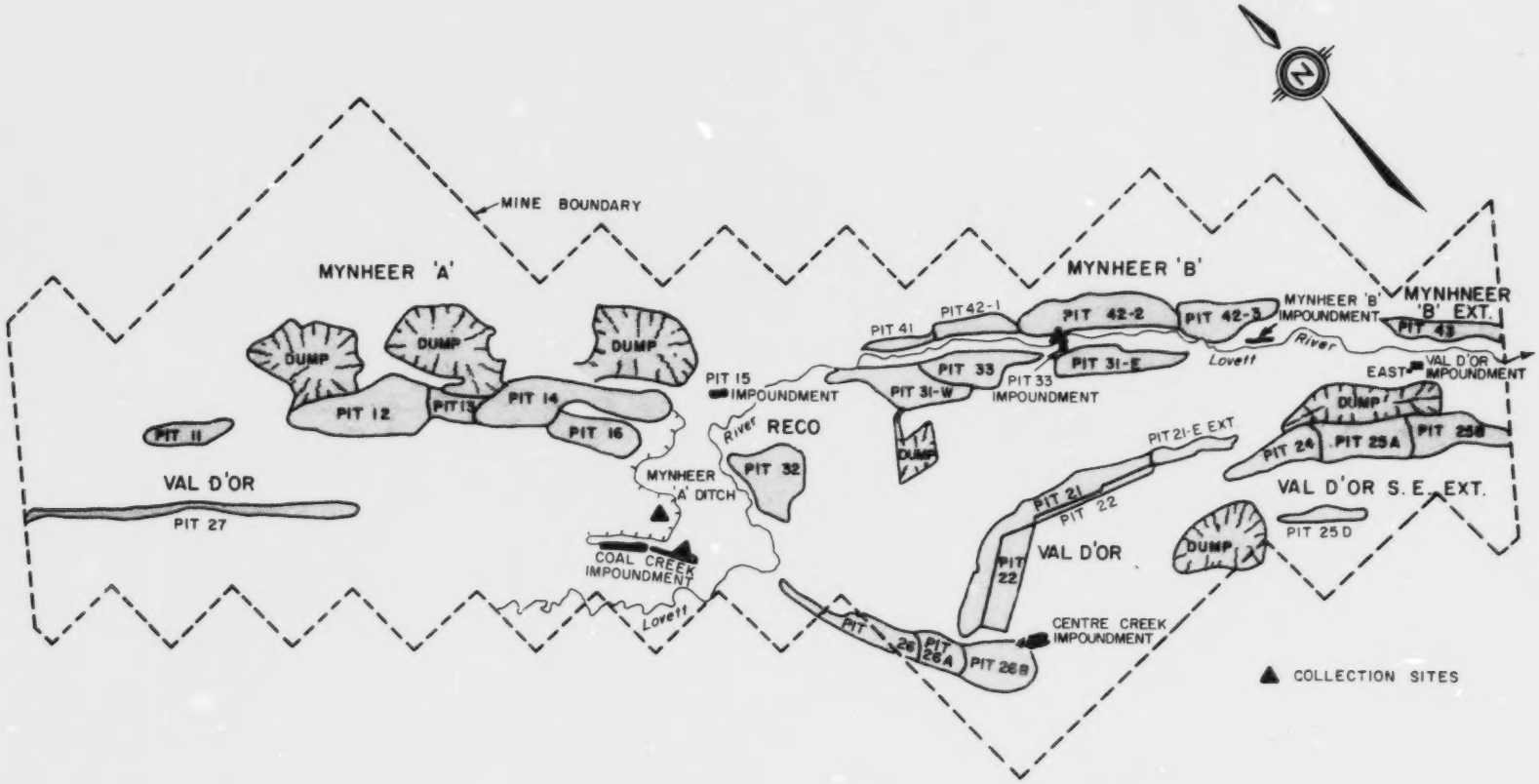


FIGURE 2. COAL VALLEY MINE SHOWING MINE PITS AND WASTEWATER IMPOUNDMENT AREAS.

The expected maximum concentrations of these combinations that would be added to raw waste water at the mine are:

i)	ferric sulfate	170 mg/ℓ
ii)	cationic 885?	20 mg/ℓ
iii)	cationic 577C	25 mg/ℓ
iv)	cationic LT31	40 mg/ℓ
v)	anionic 8184	10 mg/ℓ

The purpose of this study is to determine the toxicity to fish of effluents and flocculant combinations at maximum working concentrations. Initially rainbow trout Salmo gairdneri were exposed to various concentrations of flocculants at 4 and 15°C. At the time of death or at the termination of the study, selected test animals were subjected to pathological examination to determine the cause of disease or death.

2. LITERATURE REVIEW

2.1 Background

The mining industry began to use synthetic, water soluble polymers as flocculating agents in 1951. These flocculants may be tailored to

meet many needs, including effluent treatment, recycle water clarification, beneficiation, and treatment of complex mixtures of municipal and industrial wastes. Selection of a flocculant depends on optimizing the cost-to-performance ratio, consistent with environmental standards. Some of the most important factors in determining the suitability of a flocculant are: flocculant availability, handling, storage, reproducibility, design of existing waste treatment systems and toxicity in receiving waters. Although several hundred different kinds of flocculants are available, most of these compounds have a similar chemical structure.

Natural substances have also been used as settling agents through historic times. The first written account on the use of lime and aluminous earth was prepared during the first century AD. Although lime and alum continued to be the most important settling agents in use up to the twentieth century, certain organic polymers, such as glue and starch also found wide use in the mineral and coal processing sectors.

Today, flocculants are classified into three categories: i) inorganic, ii) synthetic organic, and iii) natural organic polymers. Although inorganic flocculants have the largest sale volume on a weight basis, synthetic organic flocculants showed the largest sale value. The terms flocculation and coagulation are considered interchangeable by the International Union of Pure and Applied Chemists.

2.2 Mechanisms in Flocculation

The energy of interaction among particles arises from two processes: overlap of double layers (films of opposing negative and positive charges) leading to repulsion, and development of London dispersion forces which lead to the subsequent development of van der Waals forces of attraction (Figure 3). A London dispersion force is the electromotive force operating in a fluid medium containing moving colloids. Van der Waals forces are weak in nature but strong enough to coalesce subcolloids to form larger particles. The distance between colloidal particles is strongly influenced by the magnitude of the double layer, which then influences the stability of the suspension. Flocculants enhance aggregation by modifying normal energy interactions or by introducing new energy relations, thus destabilizing the suspension and influencing solid-liquid separation.

Suspensions of minerals and other hydrophobic particles are generally stabilized by surface charge. By contrast, sewage sludges and other hydrophilic particles are stabilized by solvation and by charge. Solvation is the process by which a complex ion is formed by the chemical or physical combination of a solute ion or molecule with a solvent molecule. For example, use of a polymer as a flocculating agent results in interpenetration of particles by the polymer segments, thus forcing solvent molecules out of the interpenetration volume.

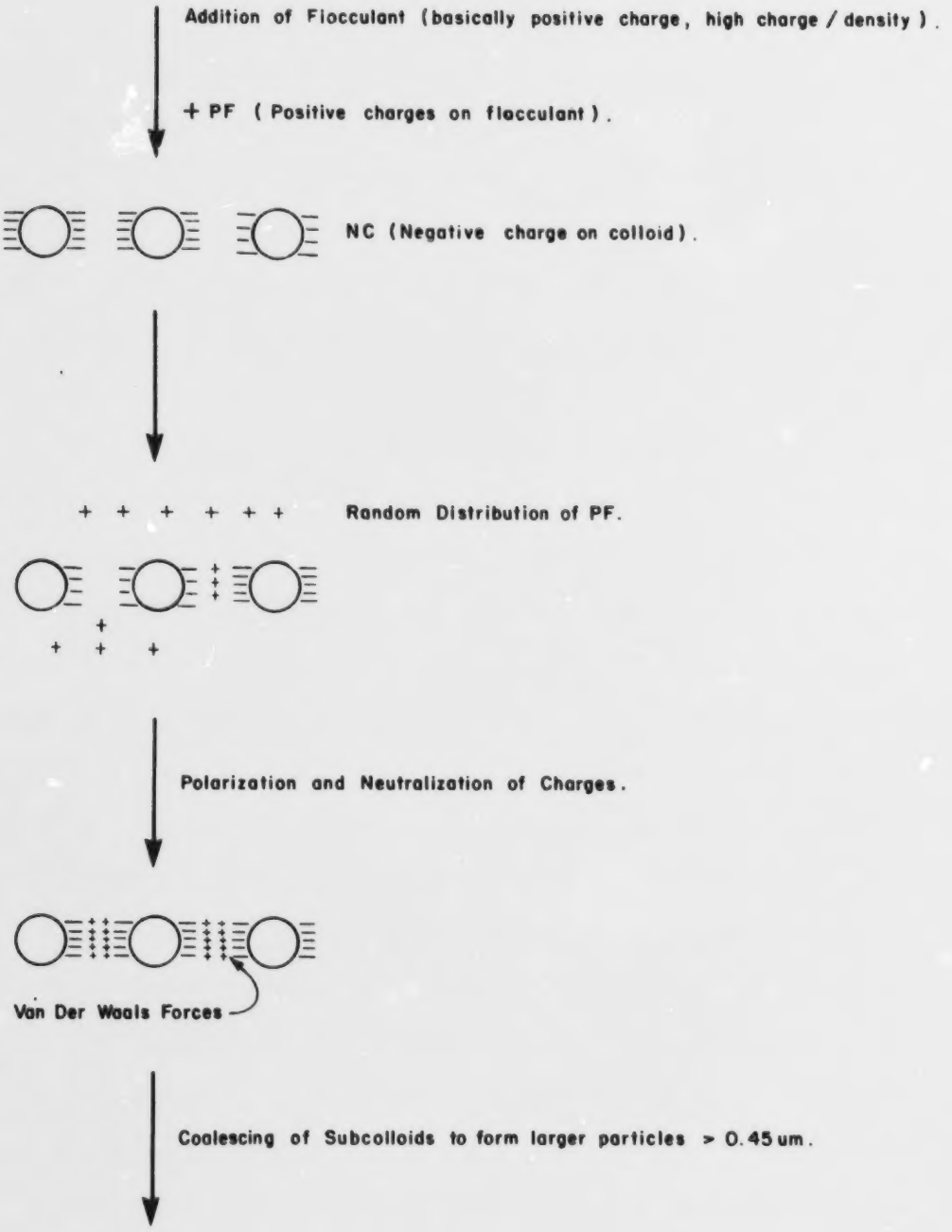

Addition of Flocculant (basically positive charge, high charge / density).
+ PF (Positive charges on flocculant).
NC (Negative charge on colloid).
Random Distribution of PF.
Polarization and Neutralization of Charges.
Van Der Waals Forces
Coalescing of Subcolloids to form larger particles > 0.45 um.

FIGURE 3. PROCESSES BY WHICH SUBCOLLOIDS COALESCE TO FORM LARGER PARTICLES.

A. Inorganic Flocculants

Many inorganic salts have hydrolyzing ions and specific ion interactions with the substrate. The hydrolysis reactions of cations involving hydroxide ions can be represented by the generalized formula

$$xZ^{+++} + y(OH^-) \qquad Z_x(OH)_y^{(3x-y)+}$$

where Z is the cation (usually Fe or Al).

The three charges on the cation are only an example and could in fact be represented by a units. A number of factors influence the nature of hydrolysis products, including pH, temperature and age of solution.

Compared to aluminum, the hydrated ferric ion is more acidic, forms stronger complexes with simple anions, and its amorphous form is less acidic, but the two show a gross similarily in their hydrolysis reactions. Sulfate ions tend to act as a catalyst in the formation of polynuclear complexes and their linkage to form a solid lattice. This means that precipitates form at lower pH values, particularly for sulfate ions compared to other anions. The most important iron derivatives used in flocculation are ferric chloride, ferric sulfate and ferrous sulfate.

The following reactions occur when ferric sulfate/calcium bicarbonate are added to water:

$$Fe_2(SO_4)_3 + 6H_2O \rightarrow 2Fe(OH)_3 + 3H_2SO_4 \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

$$3Ca(HCO_3)_2 + 3H_2SO_4 \rightarrow 3CaSO_4 + 6CO_2 + 6H_2O \quad \ldots\ldots\ldots\ldots (2)$$

$$2Fe_2(SO_4)_3 + 6Ca(HCO_3)_2 \rightarrow 4Fe(OH)_3 + 6CaSO_4 + 12CO_2 \ldots (3)$$

These reactions lead to the formation of ferric hydroxide, the coagulant. Ferric hydroxide remains insoluble over a broad pH range (5.5-11.5) compared to many other inorganic flocculants. This has led to its widespread use in industry.

Alum, the commercial term for aluminum sulfate hydrate, $Al_2(SO_4)_3 \cdot XH_2O$ is the most widely used inorganic flocculant. Since alum forms precipitates in the presence of sulfate ions at low pH, it is more effective under acidic conditions (pH 5-7) than other alum salts. Other aluminum derivatives that can be used as flocculating agents are: partially hydrolyzed aluminum chloride, poly (aluminum chloride), and sodium aluminate.

Lime consists of a group of chemicals that contain calcium and oxygen, as well as magnesium (dolomitic lime). The most important functions of lime are pH control and water softening. At the same time, it acts as a coflocculant. In fact, hydrolysis reactions of lime do not

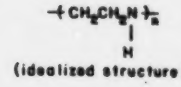
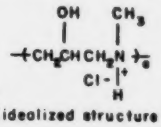
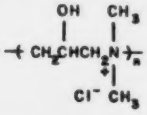
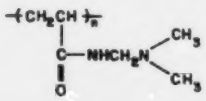
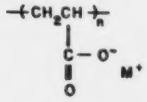
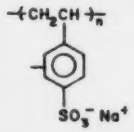
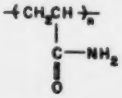
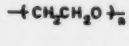
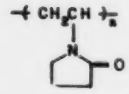
produce polymers nor is their specific ion adsorption under most industrial applications. The major types of lime are CaO, MgO, CaO-MgO, $Ca(OH)_2$, $Ca(OH)_2/MgO$, and $Ca(OH)_2$-$Mg(OH)_2$.

B. Synthetic Organic Flocculants

Use of synthetic flocculants in mineral processing is worldwide, and increasing in volume. In Canada, the coal and mineral related industries spend approximately 25 x 10^6 dollars per year on synthetics, mainly imported from the USA, and to a lesser degree, Europe and Japan (USA Minerals Yearbooks). Most synthetic flocculants are water soluble polymers, with a molecular weight (MW) of 10^3-10^6. If certain segments of the polymer are charged, it is called a polyelectrolyte. A cationic polymer, such as polyamine salts, results when the charge is positive, whereas negatively charged polymers, such as sodium acrylate, are called anionic. A polymer containing both positive and negative charges is called a polyampholyte. Nonionic polymers (for example poly (ethylene oxide) and polyacrylamide) do not contain a significant number of charged subunits and are therefore termed nonionic.

All commercially available cationic polymers contain quadrivalent nitrogen as the charged site. Cation features are independent of pH and are derived, either by protonation of primary, secondary or tertiary amines, or by generation of quaternary nitrogen grouping. Non-charged parts of the polymer may exhibit sensitivity to pH, such as hydrolysis of ester linkages.

The number of basic synthetic organic flocculant structures is relatively small (Table 1). Polyamines are a major group and include poly (alkylene polyamine), poly (hydroxyalkylene polyamine) and polyamine quaternaries. As illustrated in Figure 4, the availability of sites with cationic charge depends strongly on pH for some compounds, whereas in other cases, pH has no apparent effect on the fraction of charged sites. Other important synthetic polymers include cationic cyanamide derivatives, vinyl polymer conversion products such as cationic carbamoyl polymers and poly (2-vinylimidazolene), and vinyl addition polymers.

POLYMER	MOLECULAR FORMULA	CHARGE	COMMENTS
poly(ethyleneamine)	 (idealized structure)	cationic	homopolymer; charge density function of pH
poly(2-hydroxypropyl-1-N-methylammonium chloride)	 (idealized structure)	cationic	homopolymer; charge density function of pH
poly(2-hydroxypropyl-1,1-N-dimethylammonium chloride)		cationic	homopolymer; strongly cationic, pH-insensitive, chlorine resistant, major representative.
poly [N-(dimethylaminomethyl)-acrylamide]		cationic	solution equilibria; charge density function of pH; major commercial importance
poly(sodium or ammonium acrylate)		anionic	homopolymer and major anionic constituent with acrylamide
poly(sodium styrenesulfonate)(PSS)		anionic	homopolymer of some significance as strongly anionic, pH-insensitive flocculant
polyacrylamide (PAM)		nonionic	principal nonionic flocculant and a major constituent in copolymers
poly(ethylene oxide) (PEO)		nonionic	effective nonionic flocculant for special applications
poly(vinylpyrrolidinone)		nonionic	flocculant for special applications

From Halverson and Panzer (1980)

TABLE I. PRINCIPAL COMMERCIAL SYNTHETIC ORGANIC FLOCCULANT STRUCTURES

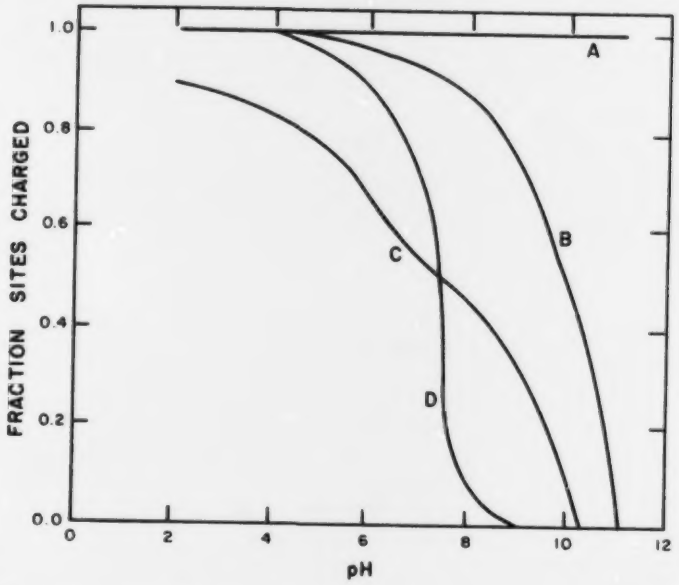


FIGURE 4. Fraction available sites with cationic charge. A, Quaternary nitrogen, poly(N,N,N-trimethylallylammonium methyl sulfate); B, poly(vinylimidazoline); C, poly(ethyleneimine), Montrek 1000; D, Mannich reaction product of poly(acrylamide).
From Halverson and Panzer (1980).

Anionic flocculants consist of two major categories: polymers containing carboxyl groups and polymers containing sulfonic acid groups. From 1 to 100% of the monomer units of anionic flocculants contribute to the charge. Since low MW polymers act as dispersants, the major commercial flocculants have a high to very high MW ($1\text{-}5\times10^6$, and $>5\times10^6$ respectively). The extent of ionization depends on pH in acidic substrates. By contrast polymers with sulfonic acid moieties retain their charge in media of low pH.

2.3 Flocculating Agents Under Investigation

Technical data on the flocculants, supplied by the manufacturer, are listed below:

A. Cationic 8852

Trade name:	Nalco 8852
Solubility in water (20°C):	100%
pH:	4.2
Density (25°C):	1.10
Freeze point:	-10°C
Recommended application rate:	1-20 mg/L

Source: Alchem Technical Bulletin (Burlington, Ontario)

B. Cationic 577C

Trade name:	Magnifloc 577C
Solubility in water (20°C):	100%
pH:	5-7

Density (25°C): 1.08-1.18

Freeze point: -18°C

Recommended application rate: 1-20 mg/L

Source: Cyanamid Technical Bulletin (American Cyanamid Co., Wayne, New Jersey, USA)

C. Cationic LT31

Trade name: Magnafloc LT31, Percol LT31

Solubility in water (20°C): 100%

pH: 2.0-4.0

Density (25°C): 1.14-1.18

Freeze point: not available

Recommended application rate: <50 mg/ℓ

Source: Allied Colloids Technical Bulletin (Mississauga, Ontario, L4V 1R9)

D. Ferric Sulfate

Trade name: Ferri-floc

Solubility in water (20°C): Not available

pH: Not available

Density (25°C): Not available

Freeze point: Not available

Recommended application rate: ~170 mg/ℓ

E. Anionic 8184

No data available

It is apparent from these data that some relevant environmental information is missing. This includes partition coefficients (water sediment, suspended solids, fish), half-life under natural conditions and in tissues, concentrations factors in fish, rate of volatilization etc. Continued, large-scale use of the flocculants with subsequent deposition into receiving waters would require the elucidation of such data.

3. MATERIALS AND METHODS

3.1 Collection of Mine Effluent

All tests were conducted initially using final effluent collected from the waste treatment ponds (Figure 2). These ponds receive different flocculants in various concentrations. The decanted portion is then discharged to the Lovett River. A second series of tests were conducted on influent prior to its retention in the waste treatment ponds (Figure 2). A selected series of control bioassays were also conducted using treated municipal (Vegreville) water.

All waste waters were collected directly from the mine using an effluent pump. Final effluent was taken from the middle of Pit 33 and Coal Creek Impoundment approximately 20 cm below the surface. The floc-sediment complex on the bottom was left undisturbed. The mine eventually dredges this material and deposits it elsewhere on the site. Some ponds are also reclaimed leaving the floc-sediment complex undisturbed. Untreated influent was sampled from Mynheer Ditch A and

Coal Creek. All material was then transported to our laboratory in 25 gallon drums lined with inert plastic. The samples were stored in the dark at 2-4°C for up to 2 weeks prior to use.

3.2 Toxicity Tests

All tests were conducted using rainbow trout (Salmo gairdnerii) measuring 2.7 to 6 cm in length and 0.3 to 2 g live weight. The fish were obtained as eggs from the Mount Lassen Trout Farm (California) and reared in the laboratories of the Environmental Protection Service, Edmonton. Table 2 gives the chemistry of water used in egg rearing. All parameters fall well within levels that are suitable for rearing eggs (McGuinness, 1982). Fry were transported to the Alberta Environmental Centre and allowed to acclimate at least 2 weeks prior to experimentation. Physico-chemical conditions in the maintenance water were suitable for rearing fish (Table 3; McGuinness, 1982).

Toxicity was measured using 96 h lethal concentration (LC) tests. All experiments were conducted at 15±1°C and 4±1°C to simulate summer and near-winter conditions respectively. A number of different combinations of water mixtures of flocculants were examined. It was not possible to determine the actual concentration of flocculant in each test. Although it has been assumed that exposure concentration was near the applied dosage, there may have been some sorption to the walls of the container. This in turn would reduce exposure concentration (Table 4). Each test procedure followed a number of steps:

1. Six polyethylene bags were rinsed three times with the dilution water to be used to remove potential contaminants, and were then checked for holes.
2. The dilution water was prepared using a clean polyethylene pail reserved for that purpose. Dilution volume was either 20L or 40L.
3. The temperature, pH, conductivity and dissolved oxygen level of each chamber was determined.
4. Ten fish were used in each test chamber; these fish were not fed throughout the experiment.
5. Observation for potential mortality were made at the start of the test and at 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, 8 hours, 24 hours, 48 hours, 72 hours and 96 hours. Fish were considered dead when there was no respiratory or other movement, and no response to gentle prodding.
6. Control chambers, using only the dilution water, were also maintained throughout the 96 h experimental period.

Table 2. Physico-chemical characteristics of water used to rear rainbow trout eggs. Concentrations expressed as averages (mg/ℓ) during rearing period*.

Temperature (°C)	11.5-12.5	Calcium	26
Dissolved Oxygen	8.4-8.9	Chromium	<0.05
pH	7.6-7.9	Copper	<0.02
Chloride	3.4	Iron	<0.02
Total hardness	99	Lead	<0.0005
Calcium hardness	66	Magnesium	81
Magnesium hardness	33	Nickel	<0.008
Total alkalinity	34	Potassium	0.8
Sulfate	68	Sodium	3.9
Conductivity	210	Zinc	0.03

*Chemical parameters based on single determination provided by Environmental Protection Service (Edmonton).

Table 3. Physico-chemical conditions of water used to rear rainbow trout fry in treated municipal water. Concentrations expressed as mg/ℓ.

Temperature (°C)	12.5-15.0	Calcium	12-15
Dissolved Oxygen	8.0-9.5	Iron	<0.02
pH	7.0-7.9	Magnesium	5
Chloride	2	Potassium	0.3-0.4
Total Hardness	51-58	Sodium	3-13
Total Alkalinity	42-49	Silica	1.4-1.5
Sulfate	26-28	Nitrite	<0.05
Conductivity	136-154		

Table 4. Concentration of flocculants used in different dilution waters.

Dilution Water -- Untreated Influent

Compound	Temperature (°C)	Applied Dosage (mg/ℓ)
Anionic 8184	15	300, 250, 200, 150, 100
	4	500, 400, 300, 250, 200, 150, 100
Ferric Sulfate	15	125, 100, 75, 50, 25
	4	500, 400, 300, 200, 125, 100, 75, 50, 25
Cationic 577C	15	40, 25, 10, 5, 1
	4	125, 100, 75, 50, 40, 25, 10, 5, 1
Cationic 8852	15	75, 40, 25, 10, 5, 1
	4	125, 100, 75, 50, 40, 25, 10, 5, 1
Cationic LT31	15	40, 25, 10, 5, 1
	4	125, 100, 75, 50, 40, 25, 10, 5, 1

Dilution Water -- Final Effluent

Compound	Temperature (°C)	Applied Dosage[a] (mg/ℓ)
Anionic 8184*/Ferric Sulfate	15	300, 250, 200, 150, 100**
	4	410, 380, 350, 320, 290, 260, 230, 200, 170**
Anionic 8184*/Cationic 577C	15	40, 25, 10, 5, 1**
	4	25, 20, 15, 10, 5, 1**
Anionic 8184*/Cationic 8852	15	40, 25, 10, 5, 1**
	4	40, 37, 34, 31, 28, 25, 10, 5, 1**
Anionic 8184*/Cationic LT31	15	40, 30, 20, 10, 1.0, 0.8, 0.6, 0.4, 0.2*
	4	40, 30, 20, 10, 1**

Table 4 Cont'd. Concentration of flocculants used in different dilution waters.

Dilution Water -- Treated Municipal Water

Flocculant	Temperature (°C)	Applied Dosage[a] (mg/ℓ)
Anionic 8184*/Ferric Sulfate	15	300, 250, 200, 150, 100, 75, 50, 25, 10, 1**
	4	300, 250, 200, 150, 100, 75, 50, 25, 10, 1**
Anionic 8184*/Cationic 577C	15	40, 25, 10, 5, 1**
	4	40, 25, 10, 5, 4, 3, 2, 1**
Anionic 8184*/Cationic 8852	15	40, 25, 10, 5, 1**
	4	40, 25, 10, 5, 4, 3, 2, 1**
Anionic 8184*/Cationic LT31	15	40, 30, 20, 10, 1.0, 0.8, 0.6, 0.4, 0.2**
	4	40, 30, 20, 10, 1**

*Anionic 8184 applied at 10 mg/ℓ

[a]Applied dosage of Ferric Sulfate and cationic flocculants

**Histopathological examination done on subject fish

3.3 Histological Preparation of Fish Tissues

A representative number of fish were collected from each flocculant combination indicated in Table 4. The abdomen was slit open and immediately fixed in Bouin's solution for 24 hours and then transferred into 70% ethanol until trimming. The whole fish was processed, embedded in paraffin and sectioned for light microscopy. Five to six micron tissue sections were stained with routine hematoxylin and eosin (H&E) staining technique. Gills, heart, oral cavity, eye, brain, spinal cord, liver, pyloric caeca, intestine, pancreas, spleen, kidney, gonads and skin were examined from each fish.

4. RESULTS

4.1 Acute Toxicity

In total, 26 control bioassays were conducted using final effluent at 15° and 4°C. This is decanted material from the mine, with no experimental flocculant added. In all of these tests, there was no mortality to any fish after 96 h. Hence, final effluent discharged directly to the Lovett River was not acutely toxic to fish.

Using untreated influent as the dilution water, the 96 h LC_{50} of Anionic 8184 was >300 mg/ℓ (Table 5). Ferric Sulfate was also mildly toxic whereas the 96h LC_{50} of the Cationic flocculants was >40 to 75 mg/ℓ. The combination of Anionic 8184 and Ferric Sulfate was mildly

toxic (LC_{50}>250 mg/ℓ) in influent (Table 5). Combinations of Anionic 8184 with the Cationic flocculants were more toxic, with the 96h LC_{50}'s ranging from 1 to 40 mg/ℓ.

In all cases, the flocculants were less toxic in treated (by settling only) influent than treated municipal water due to the binding and subsequent precipitation of flocculants in the treated effluent. Temperature had no measurable effect on all test results.

Based on these findings, the maximum dosages that can be applied without inducing acute toxicity in fish are:

anionic 8184	300 mg/ℓ
ferric sulfate	125 mg/ℓ
cationic 8852	50 mg/ℓ
cationic 577C	40 mg/ℓ
cationic LT31	40 mg/ℓ

These dosages are based on static laboratory studies and do not necessarily apply to conditions at the mine or in a flow through laboratory study.

Table 5. Acute toxicity (96h LC_{50}) of flocculants using different dilution waters.

Untreated Influent

Flocculant	Temperature °C	LC_{50} - 96 h mg/ℓ
Anionic 8184	15	>300
	4	>500
Ferric Sulfate	15	>125
	4	400-500
Cationic 577C	15	>40
	4	75-100
Cationic 8852	15	>75
	4	50-75
Cationic LT31	15	>40
	4	50-75

Treated Effluent

Flocculant	Temperature °C	LC_{50} - 96 h mg/ℓ
Anionic 8184/Ferric Sulfate	15	250-300
	4	380-410
Anionic 8184/Cationic 577C	15	10-25
	4	10-15
Anionic 8184/Cationic 8852	15	10-25
	4	<40
Anionic 8184/Cationic LT31	15	1-10
	4	10-20

Table 5 Cont'd. Acute toxicity (96h LC_{50}) of flocculants using different dilution waters.

Treated Municipal Water

Flocculant	Temperature °C	LC_{50} - 96 h mg/ℓ
Anionic 8184/Ferric Sulfate	15	75-100
	4	50-75
Anionic 8184/Cationic 577C	15	1-5
	4	5-10
Anionic 8184/Cationic 8852	15	1-5
	4	10-25
Anionic 8184/Cationic LT31	15	1-10
	4	1-10

4.2 Pathological Effects

Skin, gills, heart, oral cavity, eye, brain, spinal cord, liver, pyloric caeca, intestine, pancreas, spleen, kidney and gonads were examined from each fish.

A. Normal Tissues

A brief outline with microphotographs of the normal fish skin and gill histology follows (Figures 5-17).

The skin may be naked or scaly. Scales are enclosed in dermal scale pockets and are arranged in an imbricated pattern. The main layers of the skin are the epidermis, basement membrane and dermis. The epidermis is composed of stratified squamous epithelium which varies in thickness depending on the site, age and species of the fish. Mucus-secreting cells are found in the epidermis in variable numbers. The dermis is composed of two layers: stratum spongiosum and stratum compactum. Pigment cells (chromatophores) are seen in the dermis (Figures 5 and 6).

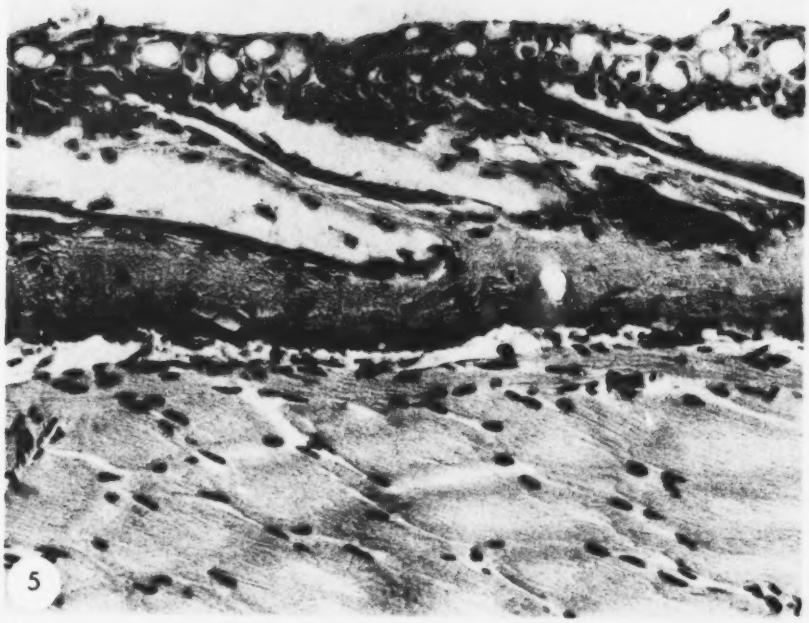
Two sets of gill arches are found on each side of the head. From each arch, a large number of long thin filaments called the primary lamellae project from the arch. The surface area of the primary lamellae is increased further by the formation of regular semilunar folds across its dorsal and ventral surface -- the secondary lamellae. The gill arch and the primary lamellae are covered by epidermal type cells. The secondary lamellae are covered with a single layer of simple

squamous epithelium on either side. Between these two thin layers are alternating pillar cells and blood capillaries (Figure 7).

Mild lesions were seen in a few of the control rainbow trout gills, characterized by diffuse hypercellularity with focal shortening and fusion of the secondary lamellae (Figure 8).

Figure 5. Photomicrograph of normal fish scaly skin showing epidermis, dermal scale pockets, dermis and muscle. X358 H&E.

Figure 6. Photomicrograph of normal naked skin showing epidermis with mucus-secreting cells, dermis and muscle. X224 H&E.



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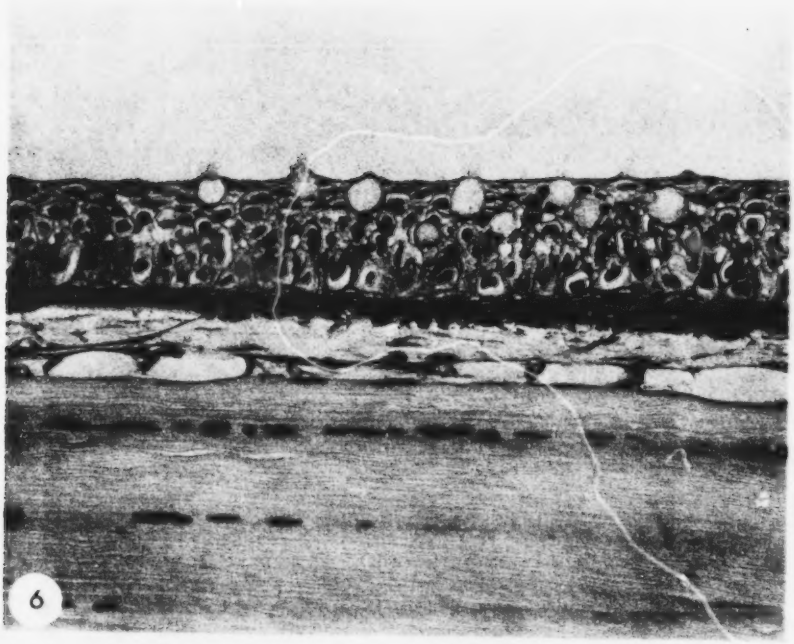

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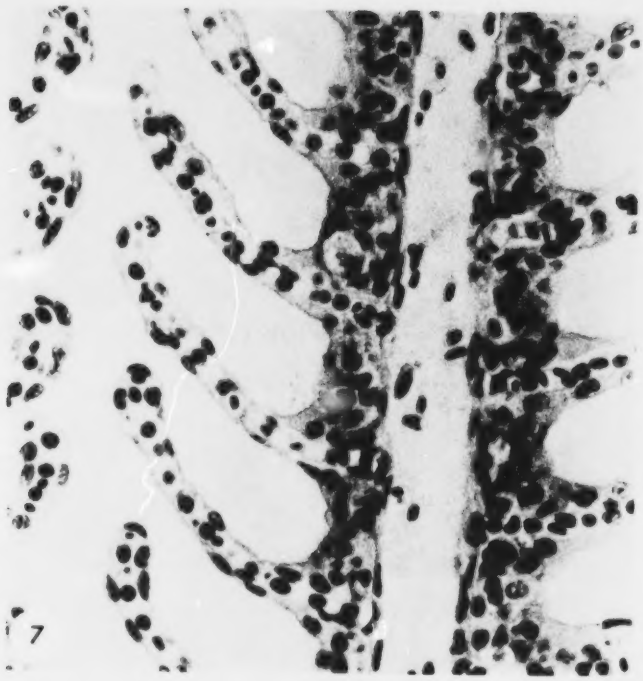
Figure 7. Photomicrograph of gill lamellae from a control fish showing arrays of delicate secondary lamella originating from a primary lamella. X280 H&E.

Figure 8. Photomicrograph of gill lamellae from a control fish showing arrays of slightly hypercellular secondary lamellae with focal fusion and proliferation of the epithelial cells (Arrow). X280 H&E.



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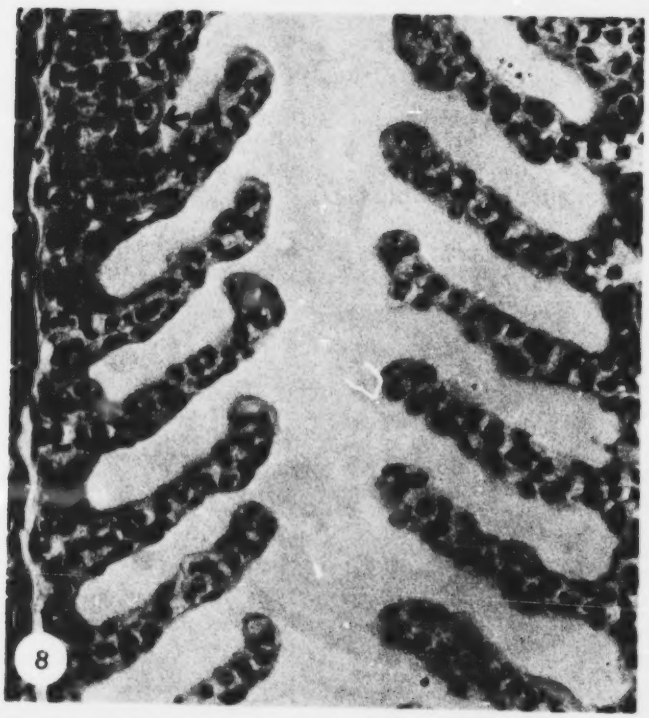

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Figure 9. Photomicrograph of normal fish heart showing the muscular ventricle and portion of the atrium. X61 H&E.

Figure 10. Photomicrograph of normal fish eye showing the conjunctiva, cornea, iris, lens, retina, sclera and scleral cartilage. X34 H&E.

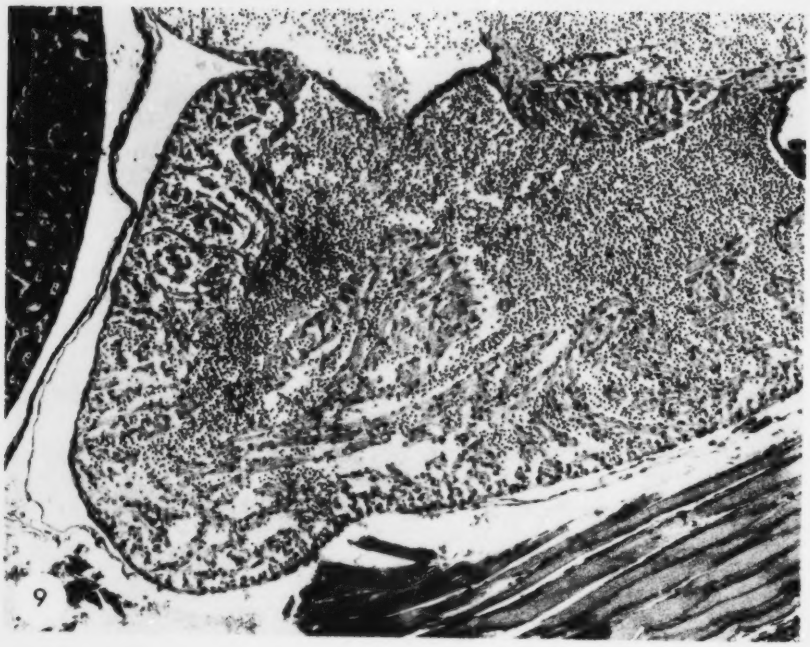

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Figure 11. Photomicrograph of normal fish brain showing portion of the telencephalon (T), optic lobe (O), third ventricle (V) and portion of the cerebellum (C). X36 H&E.

Figure 12. Photomicrograph of normal fish liver cords of hepatic cells with sinusoids filled with nucleated erythrocytes (red cells) between the cords. X280 H&E.

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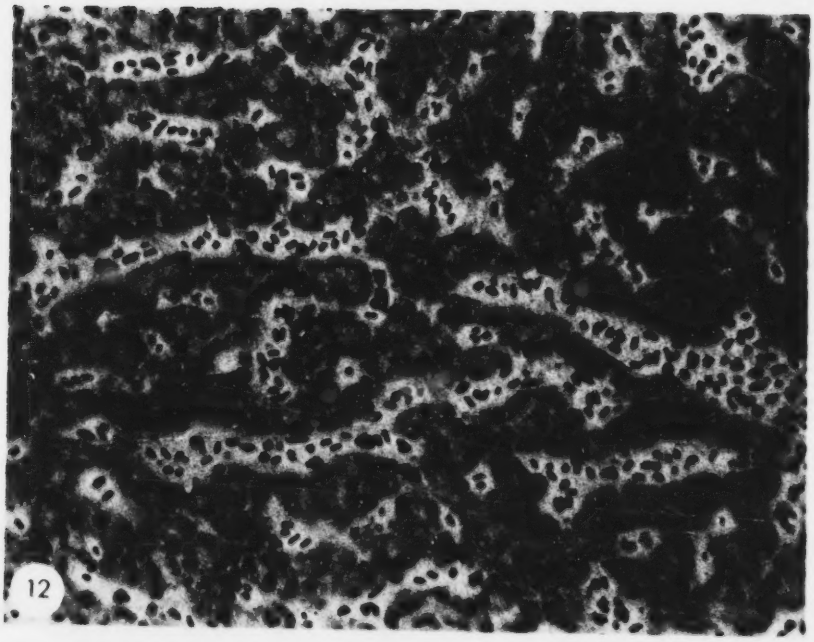

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Figure 13. Photomicrograph of normal fish pyloric caeca and pancreas. The darkely staining exocrine pancreatic acini surround an islet of Langerhans (I). X176 H&E.

Figure 14. Photomicrograph of normal fish intestine showing the mucosal epithelial lining and portion of the inner muscle layer. X168 H&E.

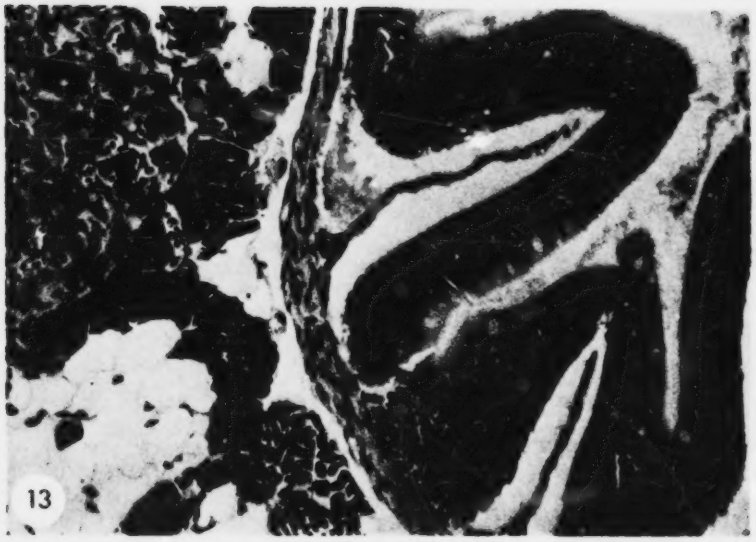
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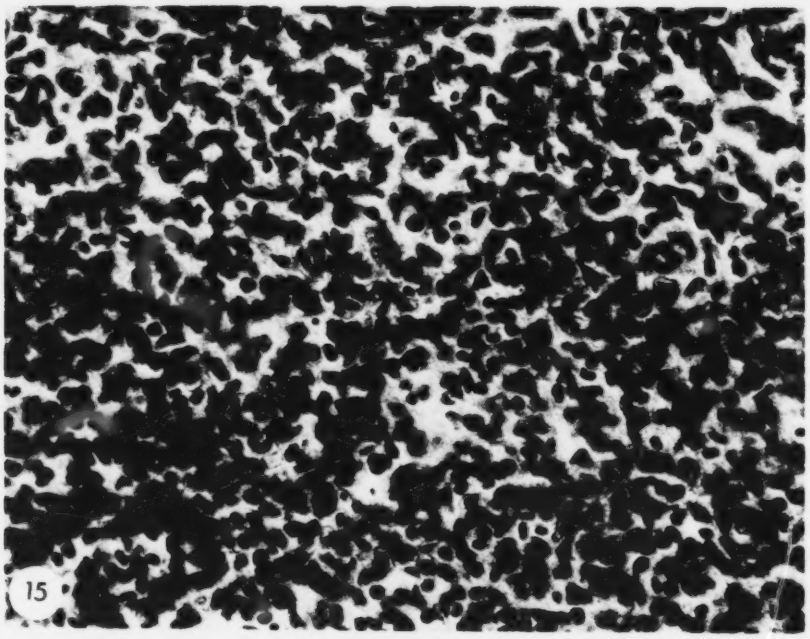
Figure 15. Photomicrograph of normal spleen showing several pale staining small ellipsoids and a splenic pulp. X224 H&E.

Figure 16. Photomicrograph of normal fish anterior kidney showing several renal tubules in a stroma of haemopoietic tissue. X280 H&E.



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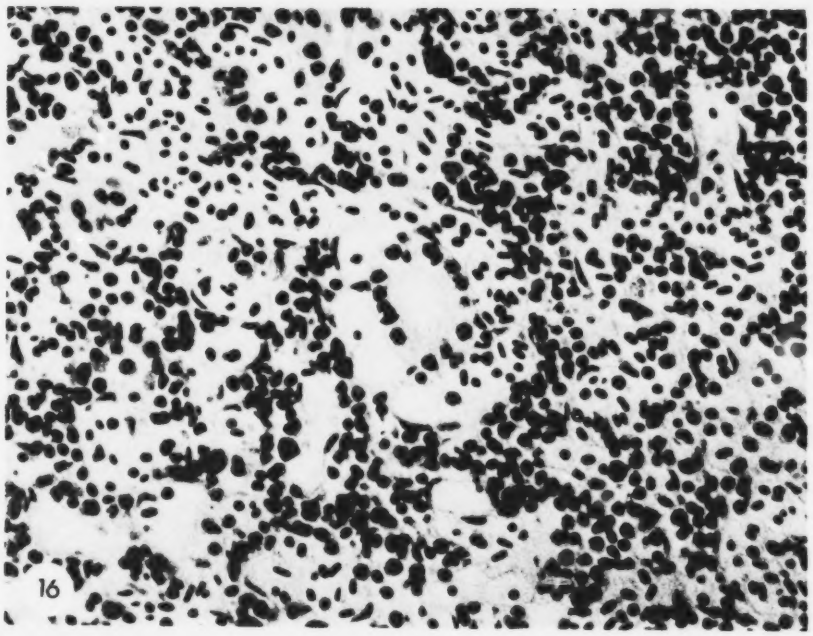

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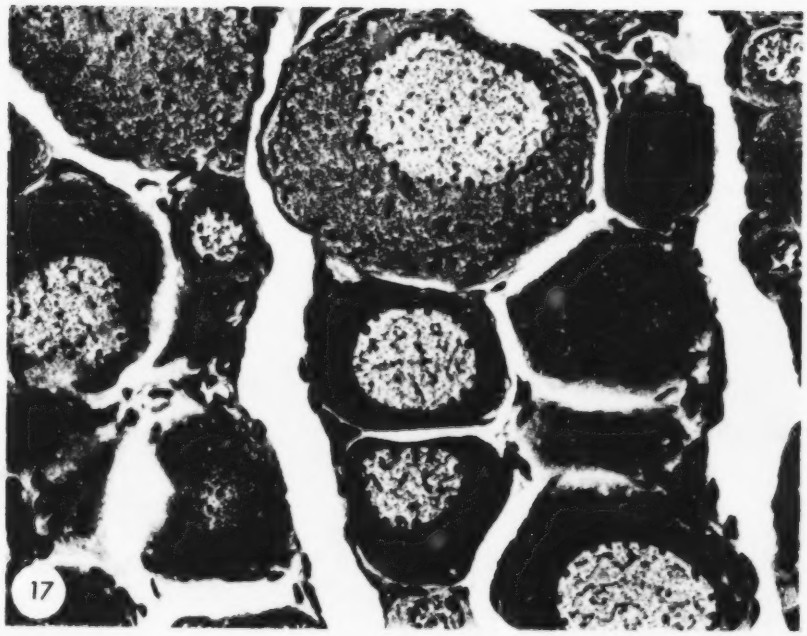
Figure 17. Photomicrograph of normal fish ovary showing several ova at different stages of development. X358 H&E.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B. Anionic 8184

No significant histopathological lesions were seen in fish exposed to the flocculant at concentrations between 6.25-100 mg/ℓ in treated water. Mild shortening and fusion of the secondary lamellae were noticed in the control and principal fish and considered as background lesions. A summary of the histological changes are given in Table 6.

Table 6. Lesions observed in fish exposed to Anionic 8184.

Treated Water

Accession No.	No. of Fish Examined	8184 Conc. mg/ℓ	Water Temp. C°	Dead (hours)	Fixed After Death (hours)	Auto-lysis	Gill Lesions	Skin Lesions	Other
938-83-A	2	Control	4	TK**	0	-	+* shortening and fusion of 2° lamellae	-	-
B	1	6.25	4	TK	0	-	+ As above	+ Thickening of the epidermis	-
C	1	12.5	4	TK	0	-	+ As above	-	-
D	1	25	4	TK	0	-	+ As above	-	-
E	1	50	4	TK	0	-	-	-	-
F	1	100	4	TK	0	-	+ Shortening and fusion of secondary lamellae	-	-

*+ Mild ++ Moderate +++ Marked ++++ Severe - None

**TK - Terminal kill

C. Anionic 8184/Ferric Sulfate

Moderate to marked gill lesions were seen in fish exposed to this combination in the treated water. Significant histopathological changes were observed at concentrations above 100 ppm of $Fe_2(SO_4)_3$ (Table 7). Similar types of lesions were seen in fishes exposed to the flocculant combination in effluent water at a concentration of 380 mg/ℓ of $Fe_2(SO_4)_3$ and above. These lesions were characterized by marked shortening, disorganization and fusion of the secondary lamellae with necrosis and sloughing of the epithelial covering (Figures 18 and 19).

Skin lesions ranged from moderate to severe in fishes exposed to the flocculants combination in treated water at concentrations between 100-300 mg/ℓ of $Fe_2(SO_4)_3$. The skin lesions of fishes in effluent water were inconsistent and varied from mild to marked changes in similar or comparable concentrations of $Fe_2(SO_4)_3$ (Table 7). The marked skin changes in both types of treatment consisted of marked vacuolation and necrosis of the epidermal epithelium with sloughing of the superficial 1/3-1/2 of the epidermis (Figures 20 and 21). In general, the skin lesions were more pronounced in the naked (nonscaly) epidermis.

Additional lesions were seen in the oral and pharyngeal regions in fishes exposed to the flocculant combination in treated and effluent types of water at concentrations between 150-300 mg/ℓ and 250-300 mg/ℓ of $Fe_2(SO_4)_3$ respectively. These

lesions were characterized by mild to moderate superficial necrosis and sloughing of the epithelial lining (Figure 22). A summary of the histological changes are given in Table 7.

Table 7. Lesions observed in fish exposed to Anionic 8184 and $Fe_2(SO_4)_3$.

Treated Water

Accession No.	No. of Fish Examined	$Fe_2(SO_4)_3$ Conc. mg/ℓ	Water Temp. C°	Dead (hours)	Fixed After Death (hours)	Autol-ysis	Gill Lesions	Skin Lesions	Other
936-83-A	1	100	15	<24	NA	++	-	++ Vacuolation and erosion of epidermis	-
B	1	150	15	<24	NA	+	-	+++ Focal vacuolation and erosion of epidermis	-
C	1	200	15	>24	>5	+++	- Autolysis	- Autolysis	-
D	1	250	15	3	NA	-	+ Fusion of 2° 2° lamellae	+++ Focal vacuolation and erosion of epidermis	-
E	1	300	15	3	NA	+	++ As above	++ As above	-
F	1	Control	15	TK	0	-	-	-	-
939-83-A	1	Control	4	TK	0	+	+ Proliferation of 2° lamellar epithelium with sloughing	-	-
B	1	100	4	<24	>5	+++	+++ Autolysis	+++ Autolysis	-
C	1	150	4	<5.25	5	++	+++ Epithelial proliferation with fusion and shortening of 2° lamellae	++ Focal necrosis and vacuolation of epidermis	++ Oral lesions
D	1	200	4	<4.5	<5	+	+++ As above	+++ As above	+ Oral lesions
E	1	250	4	<4.5	<5	+	+++ As above	++++ As above	++ Oral lesions
F	1	300	4	<4.5	<5	+	+++ As above	++++ As above	++ Oral lesions

*NA Not Available

Table 7 Cont'd. Lesions observed in fish exposed to Anionic 8184 and $Fe_2(SO_4)_3$.

Treated Water

Accession No.	No. of Fish Examined	$Fe_2(SO_4)_3$ Conc. mg/ℓ	Water Temp. C°	Dead (hours)	Fixed After Death (hours)	Autol-ysis		Gill Lesions		Skin Lesions	Other
1391-83-A	1	1	4	TK	0	-	+	Shortening and fusion of secondary lamellae		-	-
B	1	10	4	TK	0	-	+	As above		-	-
C	1	25	4	TK	0	-	+	As above		-	-
D	1	50	4	TK	0	-	+	As above	+	Vacuolation	
E	1	75	4	<23	>5	++	++	Shortening of 2° lamellae with necrosis and sloughing of epithelium	+	As above	
F	1	Control	4	TK	0	-	+	shortening and fusion of 2° lamellae		-	-

*+ Mild ++ Moderate +++ Marked ++++ Severe - None

**TK - Terminal kill

Table 7 Cont'd. Lesions observed in fish exposed to Anionic 8184 and $Fe_2(SO_4)_3$.

Effluent Water

Accession No.	No. of Fish Examined	$Fe_2(SO_4)_3$ Conc. mg/ℓ	Water Temp. C°	Dead (hours)	Fixed After Death (hours)	Autol-ysis	Gill Lesions	Skin Lesions	Other
986-83-A	1	Control	15	TK	0	-	-	-	-
B	1	100	15	TK	0	-	-	+ Focal epidermal vacuolation and necrosis	-
C	1	150	15	TK	0	-	-	+++ As above	-
D	1	200	15	TK	0	-	+ Shortening and irregularity of 2° lamellae	+++ As above	-
E	1	250	15	TK	0	-	+ As above	+++ As above	+ Oral lesions
F	1	300	15	<6	<1	+	++ As above	+++ As above	+ Oral lesions
1359-83-A	1	170	4	TK	0	-	++ Shortening and fusion of 2° lamellae. A few necrotic epithelial cells	-	-
B	1	200	4	TK	0	-	++ As above	+ Superficial epidermal vacuolation	-
C	1	230	4	TK	0	-	++ As above	-	-
D	1	260	4	TK	0	-	+ As above	+ Superficial epidermal vacuolation	-
E	1	290	4	TK	0	-	++ As above	+ As above	-
F	1	Control	4	TK	0	-	+ Shortening and fusion of 2° lamellae	+ As above	-

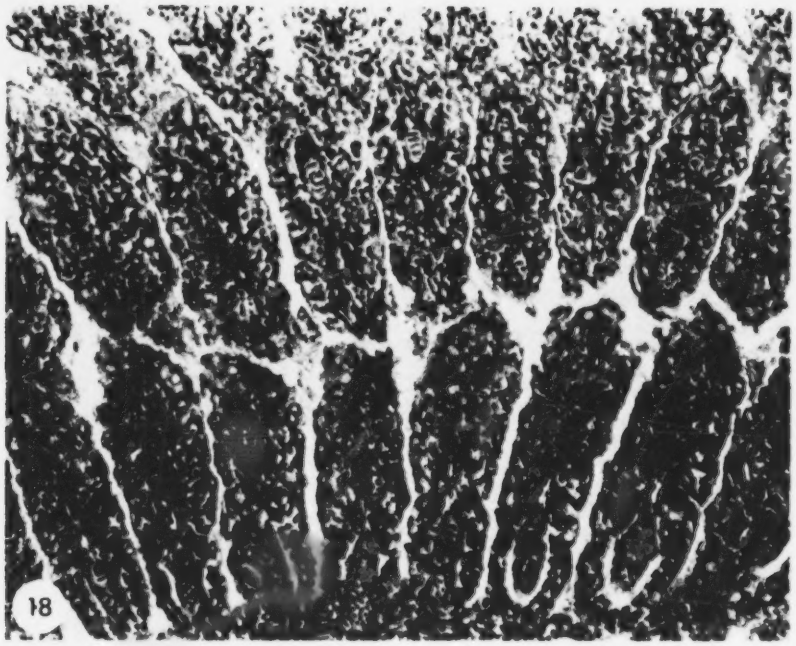
Table 7 Cont'd. Lesions observed in fish exposed to Anionic 8184 and $Fe_2(SO_4)_3$.

Effluent Water

Accession No.	No. of Fish Examined	$Fe_2(SO_4)_3$ Conc. mg/ℓ	Water Temp. C°	Dead (hours)	Fixed After Death (hours)	Autol-ysis		Gill Lesions		Skin Lesions	Other
1390-83-A	2	290	4	TK	0	-	+	Shortening and fusion of 2° lamellae	-		-
B	1	320	4	TK	0	-	+	As above	-		-
C	1	350	4	TK	0	-	+	As above	-		-
D	1	380	4	TK	0	-	++	Shortening and fusion of 2° lamellae with epithelial proliferation	+	Superficial epidermal vacuolation	-
E	1	410	4	2.75	<1	-	+++	Shortening and fusion of 2° lamellae with epithelial proliferation and necrosis	+++	Epidermal vacuolation and necrosis	-
F	1	Control	4	TK	0	-	+	Shortening and fusion of 2° lamellae	-		-

Figure 18. Photomicrograph of fish gills exposed to 8184/$Fe_2(SO_4)_3$ (380 mg/ℓ) flocculants combination in effluent water showing marked shortening and disruption of 2° lamellae. X90 H&E.

Figure 19. Higher magnification of a primary lamella to show marked shortening, fusion and necrosis of the secondary lamellae. Flocculants material and cellular debris are covering the lamellar epithelial cells. X448 H&E.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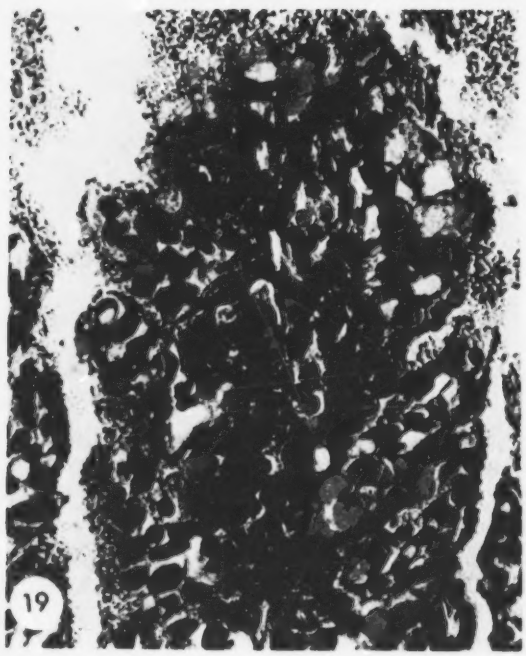
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Figure 20. Photomicrograph of fish skin exposed to 8184/$Fe_2(SO_4)_3$ (250 mg/ℓ) flocculants combination in effluent water showing necrosis and sloughing of the superficial epidermis. X280 H&E.

Figure 21. Photomicrograph of fish skin exposed to 8184/$Fe_2(SO_4)_3$ (410 mg/ℓ) flocculants in effluent water showing marked superficial vacuolation, necrosis and sloughing of the epidermal epithelium. Flocculant material are admixed with the necrotic epithelial cells (arrows). X112 H&E.

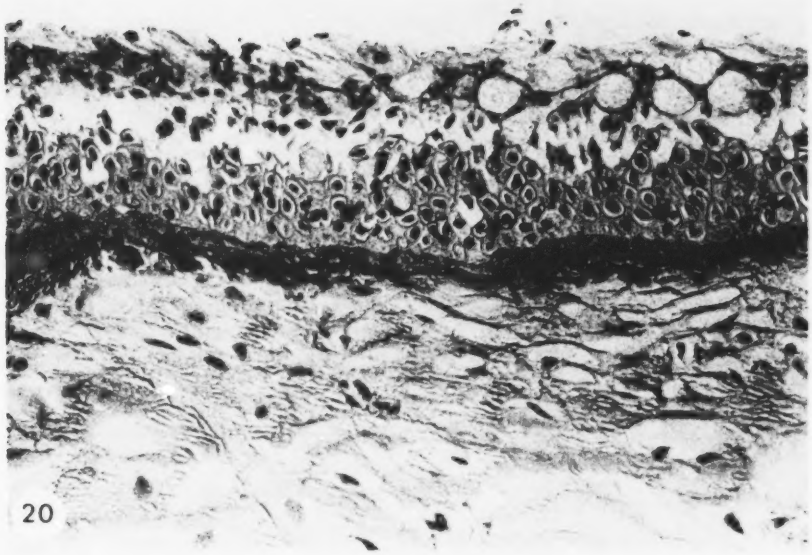

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Figure 22. Photomicrograph of fish oral mucosa exposed to 8184/$Fe_2(SO_4)_3$ (250 mg/ℓ) flocculants in treated water showing moderate superficial necrosis and sloughing of the epithelial lining. X280 H&E.

Figure 23. Photomicrograph of fish gill lamellae exposed to 8184/577C (0.5 mg/ℓ) flocculants in treated water showing marked necrosis, disruption and sloughing of the secondary lamellae. X280 H&E.

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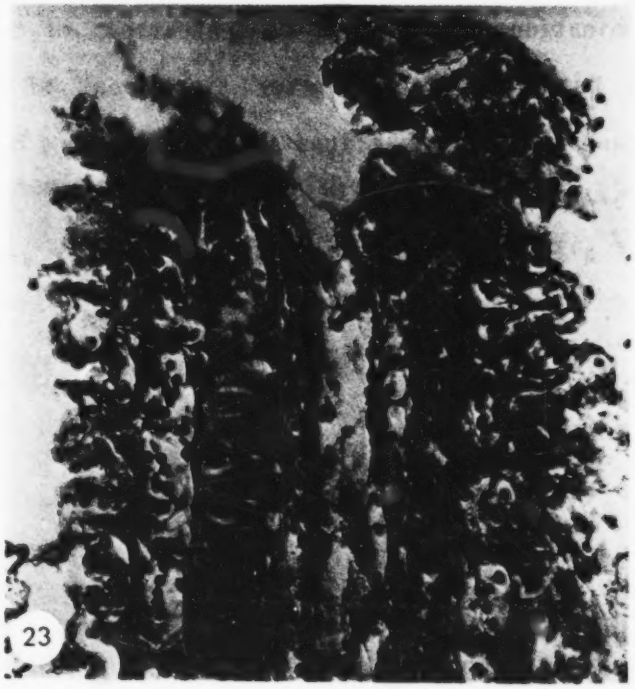

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D. Anionic 8184/Cationic 577C

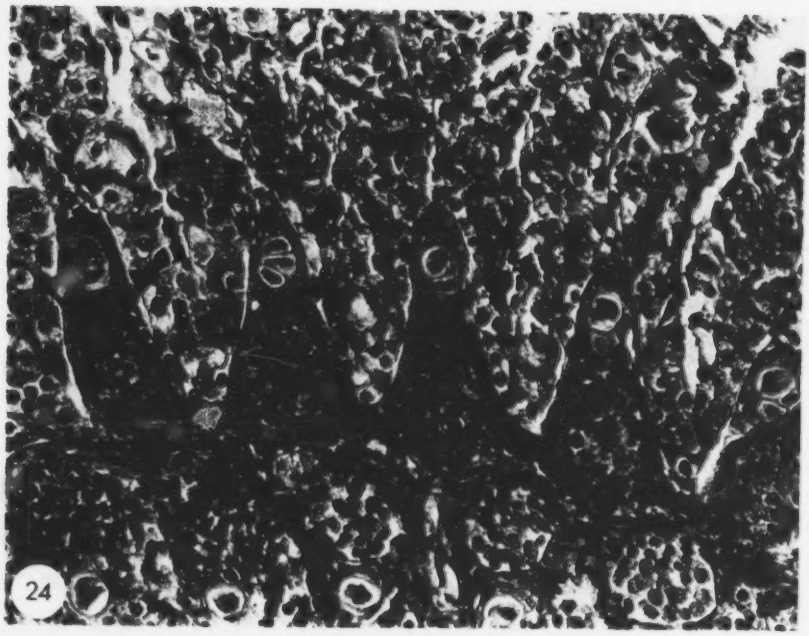
Gills lesions were seen in treated water fish exposed to the flocculant combination at concentrations between 0.5-40 mg/ℓ of 577C (Table 8). The lesions were mild to marked at concentrations between 0.5-25 mg/ℓ and severe at 40 mg/ℓ. The gill lesions in the effluent water fish were mild and significant changes were seen at concentrations between 20-40 mg/ℓ. They were characterized by marked to severe necrosis, disruption and fusion of the secondary lamellae with necrosis and sloughing of the covering epithelial cells (Figures 23 and 24).

Skin changes were generally mild to moderate in the treated and effluent water fishes exposed to the flocculants combination at different concentrations of 577C (Figure 25). One batch of treated water fishes out of four had marked cutaneous and oral lesions at concentrations between 0.5-10 ppm of 577C.

A summary of the histological changes are given in Table 8.

Figure 24. Photomicrograph of a gill arch (A) exposed to 8184/577C (40 mg/ℓ) flocculants in treated water showing marked necrosis and disruption of the primary and secondary lamellae. X280 H&E.

Figure 25. Photomicrograph of fish skin exposed to 8184/577C (40 mg/ℓ) flocculants in treated water showing mild superficial erosion and sloughing of epidermal cells. X448 H&E.



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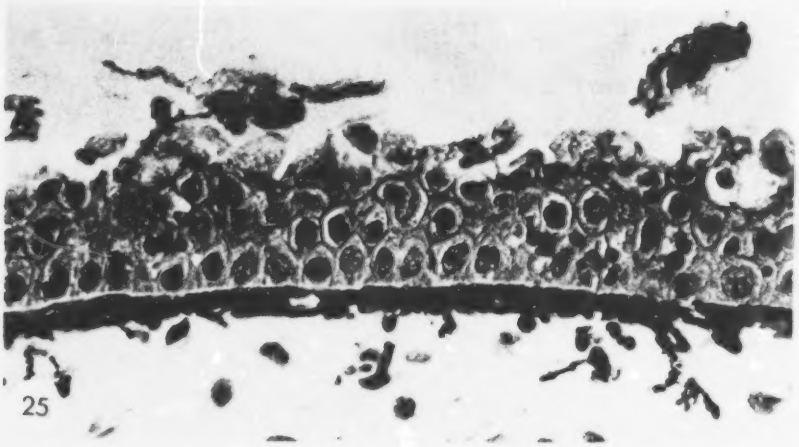

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Table 8. Lesions in fish exposed to Anionic 8184 and Cationic 577C.

Treated Water

Accession No.	No. of Fish Examined	577C Conc. mg/ℓ	Water Temp. C°	Dead (hours)	Fixed After Death (hours)	Autol-ysis		Gill Lesions		Skin Lesions		Other
1210-83-A	1	1	4	TK	0	-	+	Fusion of 2° lamellae		-		-
B	1	2	4	TK	0	-	+	As above		-		-
C	1	3	4	TK	0	-	++	Fusion and shortening of 2° lamellae		-		-
D	1	4	4	TK	0	-	++	As above		-		-
E	1	5	4	TK	0	-	++	As above		-		-
F	1	Control	4	TK	0	-	+	As above		-		-
937-83-A	1	0.5	15	<1	<1	+	+++	Lamellar shortening, necrosis and sloughing of lamellar epithelium	+++	Superficial epidermal necrosis	+++	Oral lesions
B	1	1	15	<1	<1	+	+++	As above	+++	Superficial focal epidermal necrosis	+++	Oral lesions
C	1	2.5	15	<1	<1	+	+++	As above	+++	As above	+++	Oral lesions
D	1	5	15	<½	<½	-	++++	Epithelial erosion of the lamellar epithelium	+++	As above	+++	Oral lesions
E	1	10	15	<½	<½	-	++++	Necrosis and sloughing of the 2° lamellar epithelium	+++	As above	+++	Oral lesions
F	1	Control	15	TK	0	-		-		-		-

Table 8 Cont'd. Lesions in fish exposed to Anionic 8184 and Cationic 577C.

Treated Water

Accession No.	No. of Fish Examined	577C Conc. mg/ℓ	Water Temp. C°	Dead (hours)	Fixed After Death (hours)	Autol-ysis		Gill Lesions		Skin Lesions	Other
1389-83-A	2	1	4	TK	0	-	+	Fusion and shortening of 2° lamellae		-	-
B	2	5	4	TK	0	-	++	As above	+	Epidermal thickening	-
C	2	10	4	25.5	>5	++	+++	Necrosis, lysis and fusion of 2° lamellae		-	-
D	2	25	4	1.3	<1	+	+++	Swelling and fusion of 2° lamellae	+	Diffuse erosion and vacuolation of epidermis	-
E	2	40	4	1.66	<2	-	+++	Necrosis and fusion of 2° lamellar epithelium	+	As above	-
F	2	Control	4	TK	0	-	+	Fusion of 2° lamellae		-	-

Table 8 Cont'd. Lesions in fish exposed to Anionic 8184 and Cationic 577C.

Treated Water

Accession No.	No. of Fish Examined	577C Conc. mg/ℓ	Water Temp. C°	Dead (hours)	Fixed After Death (hours)	Autol-ysis	Gill Lesions		Skin Lesions		Other
1057-83-A	1	Control	15	TK	0	-	-		++	Focal epidermal erosion with diffuse vacuolation	-
B	1	1	15	TK	0	-	-		+	Epidermal thickening	-
C	1	5	15	<2	<1	-	+++	Disruption and fusion of 2° lamellae	+	Focal epidermal erosion	-
D	1	10	15	<1	<1	-	+++	As above	+	Focal epidermal erosion	-
E	1	25	15	<1	<1	+	+++	As above	+	Epidermal vacuolation and erosion	-
F	1	40	15	<1	<1	-	++++	As above	++	As above	-

Table 8 Cont'd. Lesions in fish exposed to Anionic 8184 and Cationic 577C.

Effluent Water

Accession No.	No. of Fish Examined	577C Conc. mg/ℓ	Water Temp. C°	Dead (hours)	Fixed After Death (hours)	Autol-ysis	Gill Lesions		Skin Lesions		Other
1209-83-A	1	5	4	TK	0	-	+	Lamellar fusion and proliferation of 2° lamellar epithelium	-		-
B	1	10	4	TK	0	-	+	As above	-		-
C	1	15	4	<4	1	+	++	Disruption and fusion of 2° lamellae	+	Focal epidermal erosion	-
D	1	20	4	<4	1	+	+++	As above	+	As above	-
E	1	25	4	2.5	<1	+	+++	As above	++	As above	-
F	1	Control	4	TK	0	-	+	Fusion of 2° lamellae	-		-
1056-83-A	1	Control	15	TK	0	-		-	+	Focal epidermal erosion and vacuolation	-
B	1	1	15	TK	0	-		-	+	As above	-
C	1	5	15	TK	0	-	+	Fusion of 2° lamellae with epithelial proliferation	++	As above	-
D	1	10	15	TK	0	-		-	-		-
E	1	25	15	<3.5	<1	+	+++	Fusion and disruption of 2° lamellae	+	Vacuolation	-
F	1	40	15	<2	<1	+	+++	As above	+	Vacuolation and erosion	

E. Anionic 8184/Cationic 8852

Histopathological gill changes indicative of toxicity were seen in fishes exposed to the above flocculant combinations. The lesions were more severe and seen at lower 8852 concentrations in treated water fishes than in the effluent water fishes (Table 9). The treated water fishes developed mild to marked gill lesions after exposure to 8852 between 1-40 mg/ℓ (Figures 26 and 27). The effluent water fishes had marked gills lesions between 25-40 mg/ℓ of 8852 (Figure 28). Mild to moderate gill changes were characterized by shortening and fusion of the secondary lamellae with proliferation of the squamous epithelium (Figure 26). A slight epithelial necrosis and sloughing may or may not be seen. Marked gill changes consisted of shortening and disruption of the secondary lamellae with necrosis and sloughing of their covering epithelium (Figures 27 and 28).

The skin lesions were generally mild to moderate in the treated and effluent water types fishes. They were characterized by mild focal vacuolation and necrosis of the superficial epidermal epithelium (Figure 29).

Mild to moderate oral lesions were seen in fishes exposed to 40 mg/ℓ of 8852 in both types of water.

A summary of the histological changes is given in Table 9.

Table 9. Lesions in fish exposed to Anionic 8184 and Cationic 8852.

Treated Water

Accession No.	No. of Fish Examined	8852 Conc. mg/ℓ	Water Temp. C°	Dead (hours)	Fixed After Death (hours)	Autol-ysis		Gill Lesions	Skin Lesions	Other
1208-83-A	1	1	4	TK	0	-	+	Shortening and fusion of 2° lamellae	-	-
B	1	2	4	TK	0	-	++	As above	-	-
C	1	3	4	TK	0	-	+	As above	-	-
D	1	4	4	TK	0	-	++	As above	-	-
E	1	5	4	TK	0	-	++	As above	-	-
F	1	Control	4	TK	0	-	++	As above	-	-
1392-83-A	2	1	4	TK	0	-		-	-	-
B	2	5	4	TK	0	-		-	-	-
C	2	10	4	TK	0	-	++	Shortening and fusion of 2° lamellae, proliferation and sloughing of epithelial cells	-	-
D	2	25	4	2.5	<1	-	+++	Necrosis, sloughing and fusion of 2° lamellar epithelium	++ Focal epidermal necrosis	-
E	2	40	4	2.25	<1	-	+++	As above	++ As above	++ Oral lesions
F	2	Control	4	TK	0	-		-	-	-

Table 9 Cont'd. Lesions in fish exposed to Anionic 8184 and Cationic 8852.

Treated Water

Accession No.	No. of Fish Examined	8852 Conc. mg/ℓ	Water Temp. C°	Dead (hours)	Fixed After Death (hours)	Autol-ysis	Gill Lesions	Skin Lesions	Other
996-83-A	1	Control	15	TK	0	-	-	-	-
B	1	1	15	TK	0	-	-	+ Focal epidermal erosion	
C	1	5	15	<2	<1	+	+++ Shortening and fusion of 2° lamellae with epithelial necrosis	-	-
D	1	10	15	<3.5	<1	+	+++ As above	+ Vacuolation	-
E	1	25	15	<1	<1	+	+++ Necrosis and sloughing of 2° lamellar epithelium	+ As above	-
F	1	40	15	<1	<1	+	+++ As above	+ As above	-

Table 9 Cont'd. Lesions in fish exposed to Anionic 8184 and Cationic 8852.

Effluent Water

Accession No.	No. of Fish Examined	8852 Conc. mg/ℓ	Water Temp. C°	Dead (hours)	Fixed After Death (hours)	Autol-ysis		Gill Lesions	Skin Lesions	Other
1396-83-A	2	1	4	TK	0	-	+	Swelling and fusion of 2° lamellar epithelium	-	-
B	2	5	4	TK	0	-	+	As above	-	-
C	2	10	4	TK	0	-		-	-	-
D	2	25	4	19	>5	+++	+++	Autolysis	+++ Autolysis	-
E	2	40	4	2.8	<1	-	+++	Necrosis and sloughing of 2° lamellar epithelium	++ Focal epidermal necrosis	+ Oral lesions
F	2	Control	4	TK	0	-	+	Fusion and proliferation of 2° lamellar epithelium	-	-

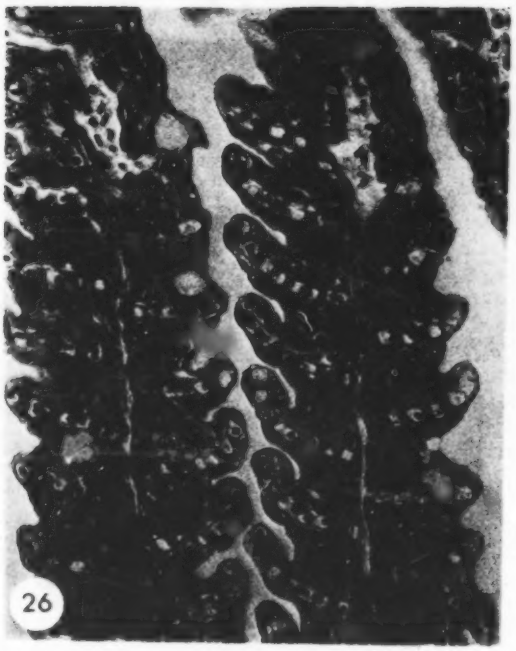
Table 9 Cont'd. Lesions in fish exposed to Anionic 8184 and Cationic 8852.

Effluent Water

Accession No.	No. of Fish Examined	8852 Conc. mg/ℓ	Water Temp. C°	Dead (hours)	Fixed After Death (hours)	Autol-ysis	Gill Lesions	Skin Lesions	Other
1205-83-A	1	25	4	<11	<5	++	+++ Erosion and disruption of 2° lamellae	-	-
B	1	28	4	<11	<5	+	+++ As above	+ Epidermal vacuolation	-
C	1	31	4	<11	<5	+	+++ As above	+ Epidermal erosion	-
D	1	34	4	<5	<1	+	+++ As above	+ Epidermal erosion	-
E	1	37	4	<11	<5	+	+++ As above	+ Focal epidermal	-
F	1	Control	4	TK	0	-	+ Fusion of 2° lamellar epithelium	-	-
997-83-A	1	Control	15	TK	0	-	-	-	-
B	1	1	15	TK	0	-	-	+ Focal epidermal erosion	-
C	1	5	15	TK	0	-	-	+ Focal epidermal vacuolation	-
D	1	10	15	TK	0	-	-	+ As above	-
E	1	25	15	TK	0	-	-	-	-
F	1	40	15	<7	<1	-	+++ Lamellar fusion with necrosis of 2° lamellar epithelium	+ Focal vacuolation and erosion	-

Figure 26. Photomicrograph of fish gill lamellae exposed to 8184/8852 (1 mg/ℓ) flocculants in treated water showing mild shortening and fusion of the secondary lamellae. X280 H&E.

Figure 27. Photomicrograph of gill lamellae exposed to 8184/8852 (25 mg/ℓ) flocculants in treated water showing shortening, fusion and necrosis of the secondary lamellae. X280 H&E.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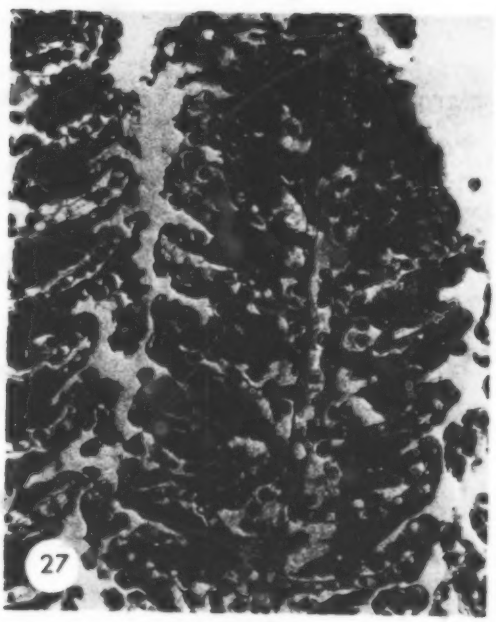
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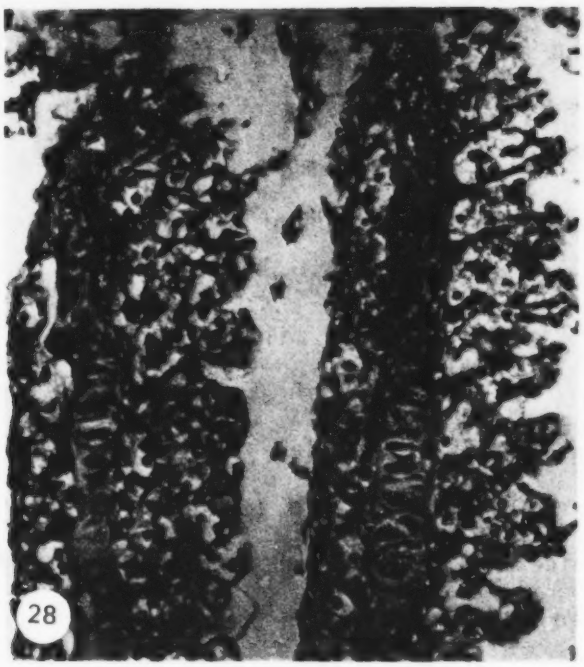
Figure 28. Photomicrograph of gill lamellae exposed to 8184/8852 (40 mg/ℓ) flocculants in effluent water showing disruption and fusion of the secondary lamellae with necrosis of the covering epithelium. X280 H&E.

Figure 29. Photomicrograph of fish skin exposed to 8184/8852 (31 mg/ℓ) flocculants in effluent water showing mild focal erosion and sloughing of the superficial epidermis. X448 H&E.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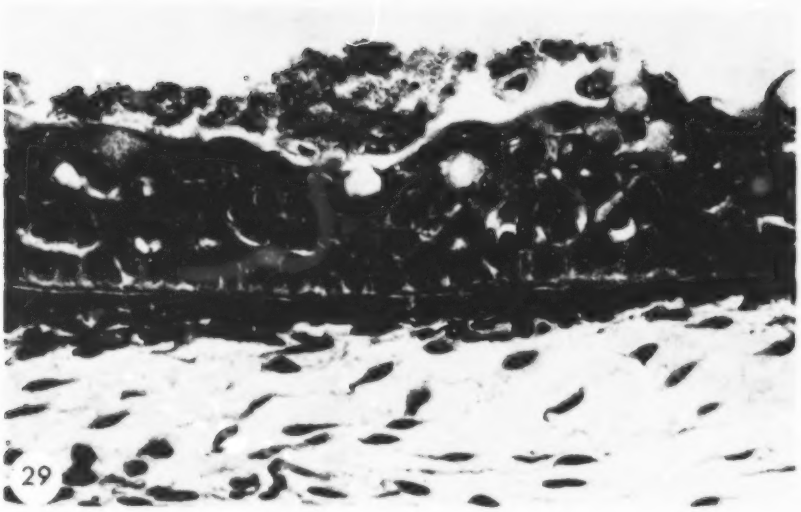
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F. Anionic 8184/Cationic LT31

Moderate to marked gill lesions were observed in the treated and effluent water fishes exposed to 20-40 mg/ℓ LT31 (Table 10). In one batch of treated water fish, gill lesions were seen at 1 and 10 mg/ℓ. The lesions consisted of marked fusion and/or disruption of the secondary lamellae with severe necrosis of the covering epithelium (Figures 30 and 31).

Skin lesions were mild to moderate and characterized by mild multifocal areas of superficial erosion and necrosis of the epidermal epithelial cells. Mild vacuolation and/or epidermal thickening were seen also. Marked multifocal areas of epidermal erosion and necrosis were seen in two fishes exposed to 40 mg/ℓ LT31 in treated and effluent types of water.

A summary of the histological changes are given in Table 10.

Table 10. Lesions in fish exposed to Anionic 8184 and Cationic LT31.

Treated Water

Accession No.	No. of Fish Examined	LT31 Conc. mg/ℓ	Water Temp. C°	Dead (hours)	Fixed After Death (hours)	Autol-ysis	Gill Lesions	Skin Lesions	Other
1397-83-A	2	1	15	TK	0	-	-	-	-
B	2	10	15	19.4	>5	+++	+++ Autolysis	-	-
C	2	20	15	1	<1	+	+++ Necrosis and sloughing of 2° lamellar epithelium	+ Thickening and vacuolation of epidermis	-
D	2	30	15	1	<1	-	+++ As above	+ Epidermal thickening and erosion	-
E	2	40	15	1	<1	-	+++ As above	+ As above	-
F	2	Control	15	TK	0	-	-	-	-
1176-83-A	1	1	4	TK	0	-	++ Diffuse fusion and shortening of 2° lamellar epithelium	++ Epidermal vacuolation	-
B	1	10	4	<8.25	>5	+	+++ Erosion, necrosis and sloughing of 2° lamellar epithelium	+ Epidermal erosion	-
C	1	20	4	<8.25	>5	+	+++ As above	+ As above	-
D	1	30	4	<2	<1	-	+++ As above	++ As above	-
E	1	40	4	<8.25	6	++	+++ As above	+++ As above	-
F	1	Control	4	TK	0	-	+ Fusion and shortening of 2° lamellae	-	-

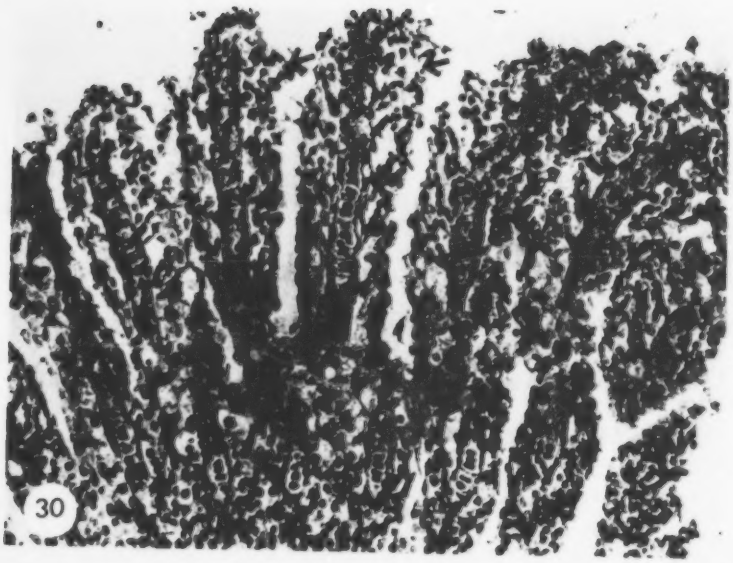
Table 10 Cont'd. Lesions in fish exposed to Anionic 8184 and Cationic LT31.

Effluent Water

Accession No.	No. of Fish Examined	LT31 Conc. mg/ℓ	Water Temp. C°	Dead (hours)	Fixed After Death (hours)	Autol-ysis	Gill Lesions	Skin Lesions	Other
1177-83-A	1	1	4	TK	0	-	-	-	-
B	1	10	4	TK	0	-	-	-	-
C	1	20	4	<8.75	>5	+	+++ Erosion and fusion of 2° lamellar epithelium	+ Epidermal erosion	-
D	1	30	4	<8.75	>5	+	+++ Erosion and fusion of 2° lamellar epithelium with sloughing	++ As above	-
E	1	40	4	<8.75	>5	+++	+++ As above	+++ As above	-
F	1	Control	4	TK	0	-	+ Shortening and fusion of 2°lamellar epithelium	-	-
1398-83-A	2	1	15	TK	0	-	-	+ Epidermal thickening	-
B	2	10	15	18	>5	++	-	+ As above	-
C	2	20	15	6	<1	+	++ Fusion and sloughing with necrosis of 2° lamellar epithelium	+ Epidermal thickening	-
D	2	30	15	6	<1	+	+++ As above	+ As above	-
E	2	40	15	3	<1	-	+++ As above	+ As above	-
F	2	Control	15	TK	0	-	-	-	-

Figure 30. Photomicrograph of gill arch exposed to 8184/LT31 (30 mg/ℓ) flocculants in treated water showing severe necrosis, disruption and sloughing of the lamellar epithelium. (Arrows) X112 H&E.

Figure 31. Photomicrograph of gill lamellae exposed to 8184/LT31 (40 mg/ℓ) flocculants in effluent water showing necrosis and sloughing of the epithelial cells with fusion of the secondary lamellae. X280 H&E.



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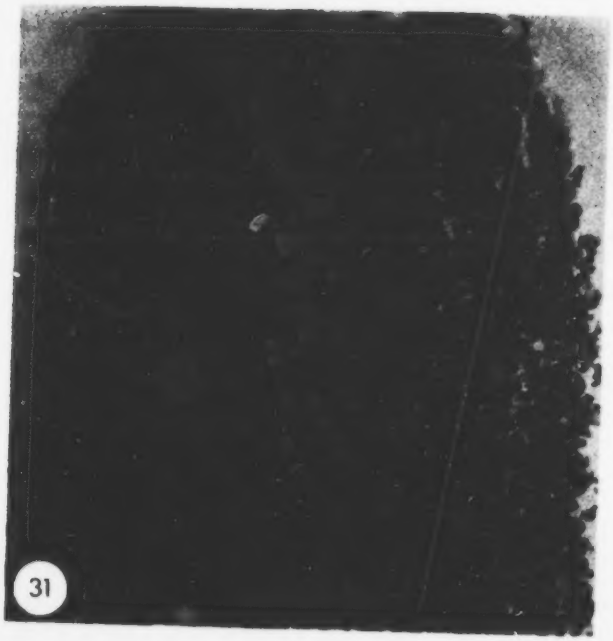

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G. Summary of Pathological Findings

1. In general, the flocculants dissolved in treated municipal water were more toxic to fish than those dissolved in effluent water.
2. Gills, skin and to a lesser degree the oral cavity, were the main tissues affected by the flocculant toxicity and naked skin was more severly affected than scaly areas.
3. The lesions were seen mostly in fish that died during the experiment, although similar lesions were seen in fish surviving 96 hours postexposure and killed terminally.

5. DISCUSSION

One of the main findings of this investigation was that physico-chemical processes within the wastewater treatment system (such as coagulation, precipitation and molecular cleavage) reduced the toxicity of flocculants. In fact, no acute or pathological effect could be found after 96 h of exposure to the final effluent. Although the effects of long term exposure on fish were not assessed, the 96 h treatments did elicit some behavioural symptoms of intoxication, particularly sluggishness of movement. We are currently determining if this symptom is mirrored by changed in enzyme activity in brain and liver and changes in blood cell counts.

The long-term fate of flocculants is not known. Any material escaping to the Lovett River would probably coalesce with suspended solids, other particulate matter, and bottom sediments. This would effectively inhibit uptake by fish, thereby limiting toxicity. Based on our pathological findings, the Cationic flocculants displayed toxic action at high concentrations. Thus a spill of free flocculants into the river might result in deleterious effects on fish through direct sorption via the gills and other membranes. However, necrosis of the epidermis and other disorders did not occur in fish exposed to final effluent; therefore, one would anticipate that flocculants in combination with wastewater would not induce these effects in the receiving waters. In addition since all of the flocculants under investigation are highly water soluble, they would be rapidly eliminated from fish tissues.

The toxicity of all flocculants was greater in treated municipal tap water, with a low suspended solid load, than effluent with a high suspended solid load. This indicated that the flocculants coalesced with suspended material, thereby reducing exposure to fish. The release of large amounts of this material, such as during a spill, would probably induce toxic effects related to suffocation, burial and mechanical abrasion. However, toxicity induced by the flocculants would be minimal.

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